Number of Pollen Grains in Brassica and Allied Genera

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Number of Pollen Grains in *Brassica* and Allied Genera

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Summary

Variation of the number of pollen grains was surveyed in *Brassica* and its allied genera.

1. In comparison between long and short stamens within a flower, differences were not observed in the number of pollen grains per anther, nor in the length of the anthers.

2. In most of self-incompatible plants in *B. campestris*, the number of pollen grains per flower was counted as 8×10^4–18×10^4 for flowers at the basal part of an inflorescence, and it decreases gradually to about half at the terminal part of the inflorescence. Inbred strains and self-compatible varieties (var. *sarson*) had 5×10^4–9×10^4 pollen grains for the basal flowers.

3. Among species with different genomic constitutions, the number of pollen grains per flower varied from 1.6×10^4 in *Erucastrum abyssinicum* to 21×10^4 in *Moricanda arvensis*. *E. abyssinicum*, *B. tournefortii* and *Diplotaxis muralis*, being ranked with lesser number of pollen grains, are self-compatible species. Though *B. napus*, *B. carinata* and *B. campestris* var. *sarson* are also self-compatible, they had a comparably high number of pollen grains. On the other hand, *M. arvensis*, *B. oleracea*, and *D. harra* were ranked as having a larger number of pollen grains. Their marked trait is that they grow in arid conditions.

Introduction

Every character of a plant must be a reflection of the evolutional process of the species. In order to keep a plant population in existence, abundant pollen grains must be produced from the flowers. An excess of pollen grains, however, may not be economical for the mass production of the plant. The number of pollen grains of a species may thus be fixed according to its breeding system and its environmental situation.

Besides, the number of pollen grains should be controlled in the breeding programme for male sterile lines.

Trying to obtain basic data for plant breeding, a survey was undertaken to know the variation of the number of pollen grains per flower among flowers within a plant, among strains within a species, and among species in a tribe *Brassiceae*.

Materials and Methods

The strains used in the investigation are shown in Table 1 divided into self-incompatibility and wild or cultivated. They were taken from genetic stocks kept in the Laboratory of Plant Breeding, Faculty of Agriculture, Tohoku University. Besides these, 26 Japanese commercial cultivars with A genome chromosomes, which were comprised of *pekinensis* and *rapa*, were also used for the survey of the variation within the species.

TABLE 1. *Material Strains*

Strain number	Species name	Cultivated or wild	Self compatibility
C103	Brassica campestris L.	C	SI
C104	B. campestris L.	C	SI
C106	B. campestris L.	C	SI
C240	B. campestris L. (pekinensis)	C	SI
C241	B. campestris L. (pekinensis)	C	SI
C333	B. campestris L. (chinensis)	C	SI
C465	B. campestris L. (rapa)	C	SI
C471	B. campestris L. (rapa)	C	SI
C503	B. campestris L. var. toria	C	SI
C633	B. campestris L. var. sarson	C	SC
C635	B. campestris L. var. sarson	C	SC
Ca109	B. carinata Braun	C	SC
Fr104	B. fruticulosa Cyrillo	W	SI
G–1	B. gravinae Ten.	W	SI
N344	B. napus L.	C	SC
Ni109	B. nigra Koch	C	SI
Ni112	B. nigra Koch	C	SI
O–3	B. oleracea L.	C	SI
T162	B. tournefortii Gouan	W	SC
Catholica-1	Diplotaxis catholica DC.	W	SI
Harra-4	D. harra Boiss.	W	SI
Muralis-1	D. muralis DC.	W	SC
Tenuifolia-1	D. tenuifolia DC.	W	SI
Abyssinicum-2	Erucastrum abyssinicum Schulz	W	SC
M. arvensis-1	Moricandia arvensis DC.	W	SI
Raphanistrum-9	Raphanus raphanistrum L.	W	SI
Raphanistrum-10	Raphanus raphanistrum L.	W	SI
Sativus-15	Raphanus sativus L.	C	SI
Sativus-18	Raphanus sativus L.	C	SI
Rugosum-10	Rapistrum rugosum All.	W	SI
Rugosum-11	Rapistrum rugosum All.	W	SI
Alba-2	Sinapis alba L.	C	SI

notes: C: cultivated, W: wild, SI: self-incompatible, SC: self-compatible.

Plants were grown in pots of 24 cm diameter in a glass house.

A standard method for counting pollen grains in the present investigation was as follows: Flower buds one day before anthesis were fixed in alcohol acetic acid (3: 1). All anthers from a flower bud were placed together in a test tube, in which an accurate 0.3 ml solution of 50% glycerine water containing 0.08% cotton blue. The solution with anthers was stirred by a 2 cm magnet. After checking to see

that no pollen grains remained in the anthers, the number of pollen grains in the test tube was estimated by a haemocytometer with 0.2 mm depth.

The haemocytometer counting was replicated 10 times on the same flower in plant level observations. The standard error was 5% or less. In the variation studies among strains and species, replications were made 4 times, its standard error being about 10%.

Results

1. Variations within a plant and within a species

Cruciferous flowers has 6 stamens, of which 2 are shorter than the other 4. Taking 24 flowers of a cultivar (C 333) in *B. campestris*, the number of pollen grains per anther was compared between the short and long stamens. Neither significant differences in number nor in their anther length were observed (Table 2).

TABLE 2. *Comparison of Anther Length and the Number of Pollen Grains Per Anther between Long Stamens and Short Stamens in a Flower*

	Averages		t-Value	Probability
	Long stamen	Short stamen		
Anther length (mm)	3.01	3.03	0.607	>0.5
Number of pollen grains per anther ($\times 10^3$)	32.3	31.5	0.630	>0.5

When a comparison was made among flowers which would open on a same day in individual plants, the standard deviation was about 10% or less to the estimated mean numbers.

Taking 2 inflorescences of a plant in several species, the number of pollen grains per flower was determined on every other day from the beginning of flowering to the end of it. Early flowers at the basal part of inflorescences produced pollen grains in abundance. The number, however, decreased gradually for flowers at the terminal part of inflorescences. The number of pollen grains of the terminal flowers was nearly half that of the flowers at the basal part. This tendency was true in the other cultivars in *B. campestris*, but not so clear in the other species with lesser amounts of pollen grains (Fig. 1).

Correlation coefficients were calculated between the pollen grain number and size characters of flower organs, using 3 plants of *B. campestris* (C 333). The highest coefficient was with anther length (X_1) being 0.866. The other coefficients with anther width (X_2), bud length (X_3), bud width (X_4), and pistil length (X_5) were 0.723, 0.618, 0.594, and 0.405 respectively. The multiple linear regression between the pollen grain number (Y) and the above 5 characters was

$$Y=(95.6X_1+61.4X_2-3.24X_3+4.43X_4-3.24X_5-165.1)\times 10^3$$

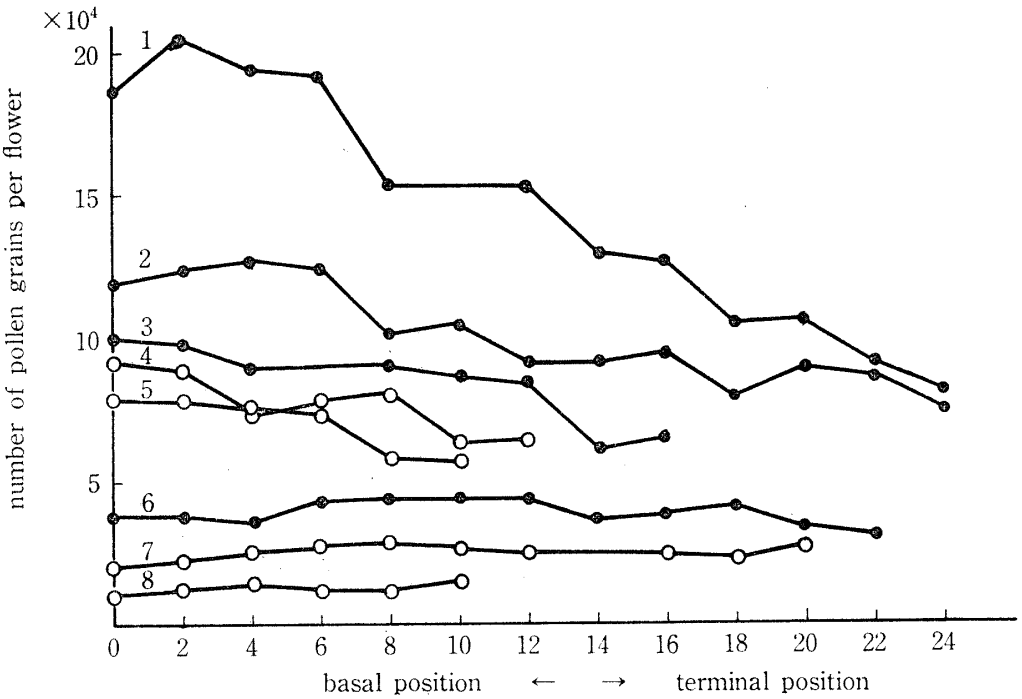

FIG. 1. Changes of the number of pollen grains in flowers on different positions along inflorescences. 1: C333, 2: C465, 3: C503, 4: C635, 5: C633, 6: Fr104, 7: M101, 8: T162

Its correlation coefficient was 0.878.

When an estimation was made from two the characters, anther length and anther width, the multiple linear regression was

$$Y=(92.3X_1+63.5X_2-172.1)\times 10^3$$

TABLE 3. *Variation of the Number of Pollen Grains Per Flower among Strains in Brassica Campestris*

Number of pollen grains per flower (×10⁴)	Number of strains	
	Commercial and wild strains	Inbred strains
5.0— 5.9	-	1
6.0— 6.9	-	1
7.0— 7.9	-	1
8.0— 8.9	3	-
9.0— 9.9	2	2
10.0—10.9	2	-
11.0—11.9	5	-
12.0—12.9	7	-
13.0—13.9	10	-
14.0—14.9	1	-
15.0—15.9	1	-
16.0—16.9	-	-
17.0—17.9	-	-
18.0—18.9	1	-

and its correlation coefficient was 0.876. It can be said that the number of pollen grains is mostly represented by the anther size.

Using 32 cultivars and genetic stocks in *B. campestris*, their variation on the pollen grain number was observed for flowers at the basal part of inflorescences. The number varied from 5×10^4 to 18×10^4. Five inbred strains which had been propagated by 4 to 10 plants in successive generations, were from 5×10^4 to 9×10^4, while the others were from 8×10^4 to 18×10^4 (Table 3). Inbreeding depression is suggested by the number of pollen grains.

2. Variation among species in tribe *Brassiceae*

Variation of the number of pollen grains was surveyed on 18 species in *Brassica* and its allied genera. The number varied from 1.6×10^4 in *Erucastrum abyssinicum* to 21.4×10^4 in *Moricandia arvensis*.

Correlation coefficients were calculated between the number of pollen grains and several characters of flowers. They were 0.751 for anther length, 0.633 for anther width and 0.362 for pistil length.

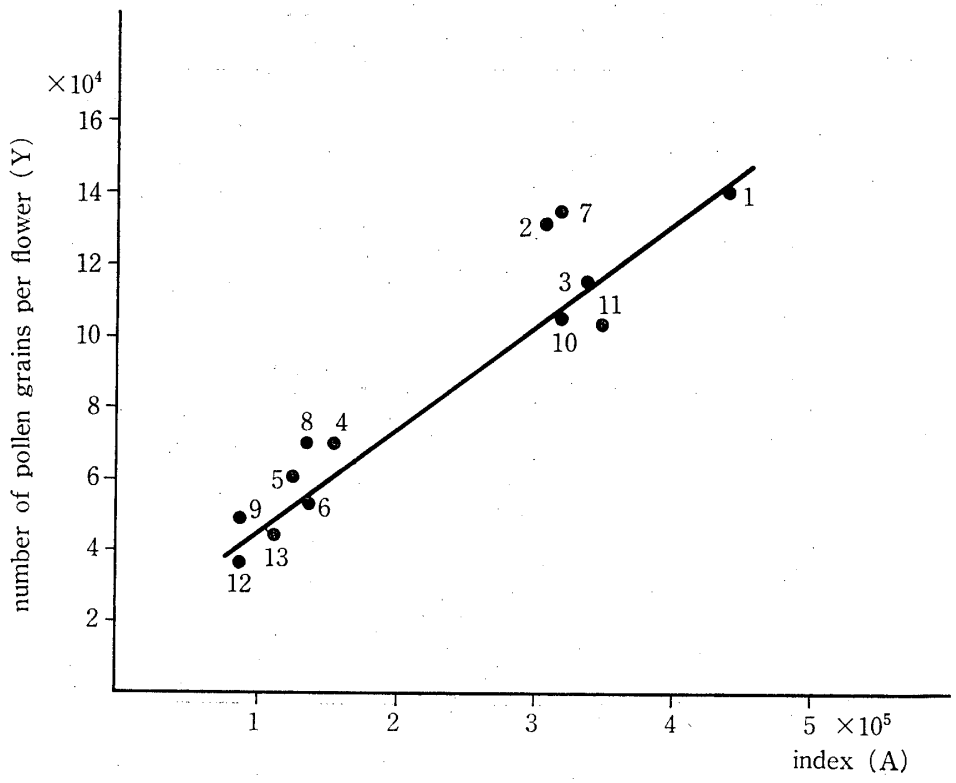

FIG. 2. Relations between the number of pollen grains (Y) and an index (A). A was determined as (anther length) (anther width)^2 / ((pollen-grain length) (pollen-grain width)^2). Linear regression formula was $Y=0.290A+0.134$. 1: *B. oleracea*, 2: *B. campestris*, 3: *B. napus*, 4: *B. carinata*, 5: *B. nigra*, 6: *B. gravinae*, 7: *D. harra*, 8: *D. tenuifolia*, 9: *D. catholica*, 10: *R. raphanistrum*, 11: *R. sativus*, 12: *R. rugosum*, and 13: *S. alba*.

Since the shape of the anther and the size of pollen grains are different for each species, a correlation coefficient was calculated between the number and an index (A). The index was calculated for each species by the following formula,

$$A = \frac{(\text{anther length}) \times (\text{anther width})^2}{(\text{pollen grain length}) \times (\text{pollen grain width})^2} .$$

The index represents an assumptive number of pollen grains estimated from the anther volume and the pollen grain volume. The correlation coefficient was as high as 0.947 (Fig. 2).

Although some of species were represented by a few strains, the species under investigation were tentatively arranged according to the number of pollen grains of them (Table 4). Three self-compatible species, *Erucastrum abyssinicum*, *B. tournefortii* and *Diplotaxis muralis* had lesser number of pollen grains per flower than the other self-incompatible species. However, the other three self-compatible species, *B. napus*, *B. carinata* and *B. campestris* var. *sarson* had a comparable number of pollen grains with self-incompatible species. On the other hand, *M. arvensis*, *B. oleracea* and *D. harra* were ranked with larger number of pollen grains than others.

TABLE 4. *Variation of Brassica Species and Its Allies on Their Number of Pollen Grains Per Flower*

No. of pollen grains per flower ($\times 10^4$)	Species
>20	M. arvensis
8–20	B. oleracea, D. harra B. campestris, B. napus*
8–10	R. raphanistrum, R. sativus
6– 7	B. carinata*, D. tenuifolia B. campestris var. sarson*
5	B. nigra, B. gravinae, B. catholica
4	S. alba
3	R. rugosum
2	D. muralis*
1– 2	B. tournefortii*, E. abyssinicum*

* self-compatible species

Discussion

For flowers at the similar growing stages, the variation of the number of pollen grains per flower was 10% or less in terms of the standard deviation. In contrast, the decrease of the number of pollen grains per flower was significant

through the flowering time, i.e. along the position of flowers on an inflorescence in *B. campestris*. High correlations were observed between the pollen grain number and organ sizes of flowers. The production of pollen grains may partly depend on the nutritional factors of the flower.

A great variation in the pollen grain number per flower was observed within a species as well as among species. As for the intra-specific variation, because of little variation of the size of pollen grains, the amount of pollen grains may be due partly to the nutritional condition and the genetic vigor of the plant.

As for the species variation, there was found a great variability for pollen grain size as well as for anther shape. A high correlation was shown between the pollen grain number and an estimated index which was calculated from the pollen grain size and anther size. The compactness of pollen grains in the anther looks to be about the same among species.

Erucastrum abyssinicum, *Brassica tournefortii* and *Diplotaxis muralis* are self-compatible species. They showed lesser number of pollen grains per flower. This may be understood in view of pollen economy, that is, the self-pollinating plants may require lesser amount of pollen grains to keep its progenies enough.

The other three self-compatible species, *B. napus*, *B. carinata* and *B. campestris* var. *sarson* produced, however, a similar amount of pollen grains to the self-incompatible ones. The former two species are allotetraploid from *B. campestris* and *B. oleracea*, and from *B. oleracea* and *B. nigra* respectively. *B. carinata*, being an endemic to Ethiopian plateau, probably have originated after the time when a cultivated kale, one of the parents, had been imported into the Ethiopian area. *B. campestris* grows as a weed in fields in the Central Plateau of Asia Minor, and its original place is supposed to be in Asia Minor or the surrounding area. It is distributed widely through Aisa (1). *B. campestris* var. *sarson* has derived from *B. campestris* as a cultivated plant in a restricted area in India. It is also considered to have originated following human cultivation. The number of pollen grains in var. *sarson* was rather less than in the other *B. campestris* cultivars. Although little information is available for *B. napus*, it is postulated that these three species have originated within human history and that their self-compatibility has appeared thereafter. It can be understood that they are still partially cross-pollinating and keep pollen grains good enough to make cross-pollination.

Besides these, another speculative point can be mentioned. *Moricandia arvensis* and *Diplotaxis harra* grow in more arid areas than do *B. nigra*, *B. catholica* and *Sinapis alba* (2). In such an arid area, plant density is lower and their flowering season is more limited due to water deficiency. The abundance of the pollen grains of *M. arvensis* and *D. harra* may be understood in view of the adaptive character required in an arid climate. A similar case is supposed for *B. oleracea*, because it grows on very steep cliffs facing the sea.

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